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14. ABSTRACT
Each major war tends to have a 'signature injury', with traumatic brain injury (TBI) associated with the Iraq war (Operation Iraqi Freedom II and Operation Enduring Freedom) due to the high incidence of personnel injured by IED (improvised explosive devices). The large gyrencephalic brain of swine is similar to humans, thus a swine model of a controlled cortical impact (CCI injury) treated by the controlled application of sub-atmospheric pressure was employed. Work completed in Year 1 showed that application of 100 mm Hg to the site of the CCI resulted in a significantly ($p < 0.01$) smaller mean contused brain tissue volume (3.44 ± 1.14 cm^3) than non-treated injuries (6.59 ± 1.76 cm^3) or 50 mm Hg treatment (9.49 ± 3.71 cm^3). Similarly, the mean intra-cranial hemorrhagic volume for treated (100 mm Hg) injuries (53.31 ± 67.81 mm^3) was significantly smaller ($p < 0.01$) than that seen in non-treated (375.75 ± 348.9 mm^3) or treated (50 mm Hg) injuries (606.84 ± 364.05 mm^3). Thus, application of 100 mm Hg sub-atmospheric pressure resulted in significantly smaller areas of injury and hemorrhage following a focal injury in a swine model compared to no treatment or application of lower levels of applied vacuum.

15. SUBJECT TERMS
Traumatic brain injury, sub-atmospheric pressure treatment, vacuum, mechanical tissue resuscitation

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Table of Contents

	Page
**Introduction……..……...…..	5**
**Body……..	5-6**
**Key Research Accomplishments…….……..	6**
**Reportable Outcomes……	7**
**Conclusion……	7**
**References…….	7**
**Appendices……	8-10**

Introduction

Each major war tends to have a 'signature injury', with traumatic brain injury (TBI) associated with the Iraq war (Operation Iraqi Freedom II and Operation Enduring Freedom) due to the high incidence of personnel injured by IED (improvised explosive devices). Our previous study in a rat model showed that Mechanical Tissue Resuscitation (MTR – the controlled application of vacuum) to the cerebral cortex following a controlled cortical impact (CCI) injury reduces brain edema and the extent of injury, modulates metabolites in injured neuronal tissues, preserves neuronal tissue, and improves functional recovery. The large gyrencephalic brain of swine is similar to humans, thus a swine model of CCI injury and MTR treatment was evaluated for future human clinical applications. Year 1 goals were to determine the optimal level of applied sub-atmospheric pressure which resulted in a smaller injury and hemorrhage volume compared to non-treated controls and less optimal levels of applied sub-atmospheric pressure.

Body

Year 1 goals stated in the Statement of Work included determination of the preferred level of sub-atmospheric pressure which results in development of the least significant injury (30 animals). Additionally, the length of time the vacuum should be applied would be started (10 animals). Only 34 animals were examined due to scheduling conflicts for imaging at the start of the year and a two week period in which the anesthesia machine in the imaging facility was broken and required repairs. Scheduling for imaging is now routine in the imaging facilities. An additional anesthesia machine is now available to prevent any further delays due to equipment failure.

All figures are appended.

Thirty four (34) female domestic swine (22-33 kg) were procured and randomly divided into groups: operated sham; CCI non-treated; CCI MTR treated - 50 mm Hg; or CCI MTR - 100 mm Hg. For creation of the CCI, animals were anesthetized and a 17 mm diameter craniotomy was performed over the right front parietal cortex. A pneumatic impactor pistol was used with the plunger parameters of 12 mm diameter, 12 mm in depth, 2.7m/s velocity, and 250ms dwell time. For MTR treatment, a sterile vacuum dressing was placed in the bony defect and either 50 mm Hg or 100 mm Hg was applied continuously for 72 hours. 72 hours post surgery, all animals were analyzed by MRI (GE Signa EchoSpeed 1.5-T scanner). Parameters analyzed included: MR sagittal T1 imaging; coronal T2 imaging; coronal MPGR (Multi-Planer Gradient Echo); Axial T2* Contrast Enhanced Perfusion (0.2 ml/Kg Magnevist contrast by power injection). All animals were euthanized and perfused with 4% para-formaldehyde through the ascending aorta 8 days post-injury. The brain was removed and postfixed in the same fixative solution overnight at 4ºC. After a PBS rinse, the brains were placed in 30% sucrose at 4°C before they were snap-frozen in O.C.T and stored at -80ºC. Coronal sections of the injured area were cut into 20 µm thick sections using a cryostat, mounted, and kept frozen until use. Sections were collected every 0.5 mm through all injured area over a total distance of 2 cm. Sections were examined after staining with haematoxylin and eosin (H&E). (Figure 1)

Total contusion injured brain volumes were measured in all coronal MR T2 weighted images as the sum of all injury areas in both groups. (Figure 2) The injured area was identified and traced as a hyperintense region ipsilateral to the injured site. There was a large area of T2 hyperintensity (edema) sometimes associated with hypointensity (hemorrhage) and herniation in T2-weighted images.

The mean contused brain tissue volume is 6.59±1.76 cm^3 in non-treated injured animals. For animals treated with MTR 100mmHg, the contused brain tissue volume decreased to 3.44±1.14 cm^3. For animals treated with MTR 50 mm Hg, the contused brain tissue volume increased to 9.49 ±3.71 cm^3. Statistical analysis showed a significant difference between injured non-treated and injured with MTR 100 mm Hg treatment ($p < 0.01$). The MTR 100 mm Hg injured brain volume was also significantly smaller than that for the MTR 55 mm Hg animals ($p < 0.01$). There is no significant difference between the MTR 50 mm Hg group compared to the non-treated injured group.

The mean hemorrhage volume in non-treated animals (375.75±348.9$mm^{3)}$ is significantly ($p < 0.01$) larger than the mean hemorrhage volume in injured MTR 100mmHg treatment (53.31±67.81 mm^3). The MTR 100 mm Hg mean hemorrhage volume is significantly ($p < 0.01$) smaller than the mean hemorrhage volume for MTR 50 mmHg (606.84±364.05 mm^3). There was no statistical difference between the mean hemorrhage volume of the non-treated injured and the MTR 50 mm Hg group.

At 8 days after injury, histopathologic results demonstrated major neuronal tissue loss and intracerebral hemorrhage in non-treated injured brains (Fig. 1 left), which confirmed that hypointense lesions seen on T2-weighted and gradient echo MR images were hemosiderin deposits of hemorrhages after injury. Less neuronal loss and hemorrhage in the injured area were observed after MTR treatment (Fig 1 right).

Additional histological and immunohistochemical analysis is ongoing, as is magnetic resonance spectroscopy analysis to determine changes in levels of excitatory amino acids and lactate.

The length of time that the sub-atmospheric pressure needs to be applied is currently being investigated. In Year 1, a default of three days of application of sub-atmospheric pressure was used. Two animals in the MTR 100 mm Hg group appeared to continue to have an increase in intracranial pressure after the 3 day treatment period based on gross observations in behavior (lethargy, decreased eating and drinking). The animals were euthanized. An increased length of treatment time is currently being examined, with a treatment duration of 5 days being investigated before the 1 day treatment (both are proposed in the Year 2 Statement of Work).

Key Research Accomplishments

- Determination of application of 100 mm Hg sub-atmospheric pressure to the site of cortical injury is superior to application of 50 mm Hg and control injury
- Statistically significantly smaller mean contused brain tissue volume with application of 100 mm Hg sub-atmospheric pressure compared to 50 mm Hg and control injury groups

- Statistically significantly smaller mean hemorrhage volume with application of 100 mm Hg sub-atmospheric pressure compared to 50 mm Hg and control injury groups

Reportable Outcomes

An abstract of the preliminary results of Year 1 was submitted, the abstract accepted, and the results were presented at the 28th Annual National Neurotrauma Symposium.

> Zheng Z., Bryant A., Argenta L., Morykwas M.: Mechanical Tissue Resuscitation Treatment Reduces Brain Tissue Volume in a Pig Traumatic Brain Injury Model. 28th Annual National Neurotrauma Symposium. Las Vegas, NV. June 14-17, 2010.

A more complete abstract of Year 1 results were submitted to the 27th Army Science Conference. It was accepted for presentation and the accompanying article will be submitted prior to the September 15th deadline.

> Morykwas M, Zheng Z, Bryant A, Argenta L: Mechanical Tissue Resuscitation Treatment Reduces Brain Tissue Volume and Intracerebral Hemorrhage in a Pig Traumatic Brain Injury Model. 27th Army Science Conference. Orlando, FL. Nov 29- Dec 2, 2010.

Conclusion

This portion of the study demonstrates that the use of mechanical tissue resuscitation (MTR) treatment of 100 mm Hg successfully reduces the extent of brain tissue injury when applied immediately post injury. MTR 100 mm Hg treated animals demonstrated a statistically significant decrease in mean contused (injured) brain volume and a decrease in intracerebral hemorrhage and neuronal tissue loss compared to non-treated control and animals treated with 50 mm Hg.

The increase in scale from our preliminary research in a rat model to the swine model, and the change from the lissencephalic rat brain compared to the gyrencephalic pig brain, did not make a difference in the success of the treatment in treating focal brain injuries.

Planned Year 2 studies of the length of treatment time have started with indications that a slightly longer treatment time may be indicated based on the gross observations of the behavior of two animals following discontinuation of MTR 100 mm Hg as described (lethargy and decreased eating and drinking).

The expectation of rapid translation of the technique to humans is still anticipated with interest from industry for commercialization of the product and technique, although no formal licensing discussions have begun.

References

N.A.

Appendices

Figure 1. Left. Injured, non-treated brain slices 8 days post injury. Right. Injured, MTR (100 mm Hg, 72 hour treatment) treated brain slices 8 days post injury. Slices are 3 mm apart through the center of CCI site. H&E. Original magnification 2X.

Figure 2. The mean total brain tissue injury volumes measured in T2-weighted MR images in traumatic brain injury pigs with/out MTR treatments. The MTR 100 mm Hg group was significantly ($p < 0.01$) smaller than the non-treated control and MTR 50 mm Hg groups. There was no significant difference between the MTR 50 mm Hg and the non-treated control group.

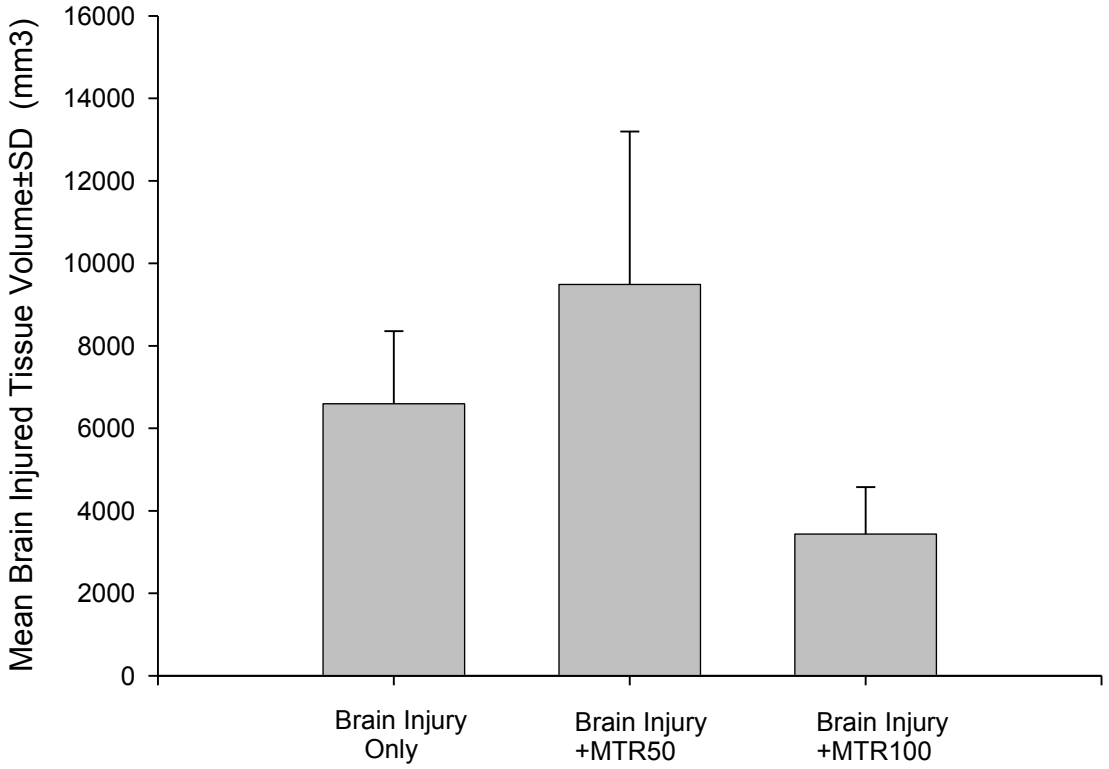

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Figure 3. The mean intracranial hemorrhage volumes measured in gradient echo MR images in pig traumatic brain injury with/out MTR treatments. The MTR 100 mm Hg group was significantly ($p < 0.01$) smaller than the non-treated control and MTR 50 mm Hg groups. There was no significant difference between the MTR 50 mm Hg and the non-treated control group.